Table S1. Sample size details for analyses on egg-spot characteristics and lymphocyte ratios (blood measurements), with geographic coordinates for each locality.

sampling information				egg-spot characteristic measurements				blood measurements
				taken from the photographs			reflectance spectrometry	lymphocyte ratio
population		latitude	longitude	all	females	males	males	males
ChL	Chitli lake	8°38'18.42"S	31°11'55.34"E	37	27	10	NA	NA
Ch1	Chitli creek 1	8°38'16.91"S	31°12'4.02"E	51	28	23	NA	NA
KaL	Kalambo lake	8°36'6.27"S	31°11'13.24"E	94	39	55	5	13
Ka1	Kalambo stream 1	8°35'35.23"S	31°11'6.18"E	53	13	40	5	6
Ka2	Kalambo stream 2	8°35'6.24"S	31°12'29.32"E	37	15	22	4	10
Ka3	Kalambo stream 3	8°35'41.59"S	31°14'50.32"E	49	23	26	8	18
LfL	Lufubu lake	8°33'36.56"S	30°43'33.79"E	29	6	23	6	9
Lf1	Lufubu stream 1	8°35'49.31"S	30°43'38.96"E	27	6	21	NA	NA
Lf2	Lufubu stream 2	8°41'9.37"S	30°33'51.90"E	36	15	21	4	6
LzL	Lunzua lake	8°44'57.13"S	31°10'21.86"E	39	16	23	4	22
Lz1	Lunzua stream 1	8°47'23.51"S	31° 8'14.33"E	52	16	36	9	10
total sample size per method				**504**	204	300	**45**	**94**

Table S2. Linear models to test for differences in egg-spot measurements based on photographs (number, relative average area, relative total area and coloration) between sexes (A) and among populations (males only) (B).

A

egg-spot characteristic	linear model
number	glmer(number ~ sex + centred_centroid_size + (1|sex_specific_population), data=data, family="poisson")
relative average area	lmer(sqrt(relative_average_area) ~ sex + (centred_centroid_size|sex_specific_population), data=data)
relative total area	lmer(relative_total_area ~ sex + (centred_centroid_size|sex_specific_population), data=data)
coloration	lmer(coloration ~ sex + centred_centroid_size + sex:centred_centroid_size + (centred_centroid_size|sex_specific_population), data=data)

B

egg-spot characteristic	linear model
number	glm(number ~ population + centroid_size, data=data, family="poisson")
relative average area	lm(relative_average_area ~ population + centroid_size, data=data)
relative total area	lm(relative_total_area ~ population, data=data)
coloration	lm(coloration^2 ~ population + centroid_size, data=data)

Table S3. Detailed results on sex-, population- and system-specific mean values (with corresponding confidence intervals) of egg-spots and the pairwise comparisons thereof. Mean values with corresponding confidence intervals for each egg-spot measurement based on photographs (number, relative average area, relative total area and coloration) and mean values with corresponding standard deviation and range for body size (standard length and centroid size) for each sex (A) and for the males of each population (B) as well as for relative total area for each system (C). Results of all pairwise comparisons for each egg-spot measurement based on photographs (number, relative average area, relative total area and coloration) between populations within lake-stream systems (D) and for comparisons of total egg-spot area between systems (E). Significance levels: *p < 0.05, **p < 0.01 and ***p < 0.001.

A

sex	egg-spot number			relative average egg-spot area (% of fin area)			relative total egg-spot area (% of fin area)			egg-spot coloration			body size			
													standard length (SL; mm)		centroid size (CS)	
	mean	lower CI	upper CI	mean	lower CI	upper CI	mean	lower CI	upper CI	mean	lower CI	upper CI	mean ± sd	range	mean ± sd	range
females	5.23	4.79	5.72	1.23	1.11	1.37	5.48	5.08	5.88	2.51	2.26	2.76	46.79 ± 7.78	27.80 - 75.40	64.67 ± 10.86	39.88 - 106.20
males	5.07	4.50	5.71	1.41	1.23	1.61	6.36	5.81	6.91	4.25	3.90	4.60	54.81 ± 12.40	33.67 - 106.42	76.14 ± 17.79	45.99 - 148.76

B

population	egg-spot number (at grandmean)			relative average egg-spot area (at grandmean, % of fin area)			relative total egg-spot area (% of fin area)			egg-spot coloration			body size			
													standard length (SL; mm)		centroid size (CS)	
	mean	lower CI	upper CI	mean	lower CI	upper CI	mean	lower CI	upper CI	mean	lower CI	upper CI	mean ± sd	range	mean ± sd	range
ChL	5.75	4.11	8.04	1.40	1.12	1.67	6.32	5.36	7.27	3.92	3.41	4.38	51.98 ± 11.66	40.14 - 75.78	72.22 ± 16.58	55.76 - 107.31
Ch1	4.64	3.36	6.41	1.54	1.29	1.80	6.46	5.31	7.60	4.65	4.26	5.01	54.21 ± 14.26	35.25 - 78.15	75.39 ± 20.04	48.74 - 108.22
KaL	5.19	3.90	6.92	1.49	1.26	1.72	6.58	5.55	7.62	4.11	3.71	4.48	55.13 ± 8.61	42.47 - 78.19	75.87 ± 11.99	58.80 - 108.37
Ka1	5.03	3.74	6.77	1.39	1.15	1.63	5.90	4.83	6.97	4.19	3.78	4.56	53.86 ± 13.59	36.09 - 89.60	74.01 ± 18.99	49.24 - 125.66
Ka2	5.05	3.64	7.01	1.45	1.19	1.71	6.25	5.10	7.41	4.25	3.81	4.64	46.98 ± 5.89	36.53 - 59.20	65.27 ± 8.44	51.08 - 82.71
Ka3	5.01	3.66	6.86	1.64	1.39	1.89	6.76	5.64	7.89	4.30	3.88	4.68	51.59 ± 12.33	33.67 - 89.88	71.22 ± 17.83	45.99 - 128.05
LfL	5.72	4.18	7.81	1.46	1.20	1.72	7.15	6.01	8.30	4.25	3.81	4.65	68.99 ± 14.38	47.17 - 102.20	97.27 ± 21.36	61.41 - 143.66
Lf1	6.75	4.22	7.93	1.67	1.41	1.93	8.09	6.93	9.25	4.50	4.08	4.88	64.32 ± 18.17	44.63 - 106.42	90.40 ± 26.02	61.93 - 148.76
Lf2	4.23	3.03	5.92	1.93	1.67	2.19	7.60	6.44	8.76	4.66	4.27	5.03	52.88 ± 7.57	41.67 - 71.23	74.48 ± 10.99	59.36 - 101.69
LzL	5.80	4.24	7.94	1.43	1.18	1.69	6.43	5.28	7.57	3.80	3.32	4.24	51.56 ± 6.38	41.58 - 67.19	71.56 ± 8.96	57.35 - 93.65
Lz1	4.14	3.04	5.63	2.04	1.80	2.28	7.21	6.13	8.29	4.54	4.16	4.88	52.27 ± 8.44	34.55 - 81.25	72.72 ± 12.18	47.48 - 115.62

C

system	relative total egg-spot area (% of fin area)			body size			
				standard length (SL; mm)		centroid size (CS)	
	mean	lower CI	upper CI	mean ± sd	range	mean ± sd	range
Chitili	6.42	5.88	6.95	53.54 ± 13.39	35.25 - 78.15	74.43 ± 18.86	48.74 - 108.22
Kalambo	6.37	5.78	6.97	52.88 ± 10.90	33.67 - 89.88	72.88 ± 15.28	45.99 - 128.05
Lufubu	7.60	6.95	8.26	62.27 ± 15.45	41.67 - 106.42	87.69 ± 22.33	59.36 - 148.76
Lunzua	6.91	6.24	7.57	52.00 ± 7.65	34.55 - 81.25	72.27 ± 10.97	47.48 - 115.62

D

population comparison	egg-spot number		relative average egg-spot area		relative total egg-spot area		egg-spot coloration	
	z-value	p-value	z-value	p-value	z-value	p-value	z-value	p-value
ChL - Ch1	1.938	0.3793	-1.062	0.9307	-0.235	1.0000	-3.531	0.0050**
KaL - Ka1	0.817	0.9834	1.570	0.6470	2.507	0.1141	-0.633	0.9964
KaL - Ka2	0.759	0.9892	0.509	0.9991	0.963	0.9585	-0.949	0.9616
KaL - Ka3	0.728	0.9916	-1.905	0.4041	-0.624	0.9967	-1.380	0.7787
Ka1 - Ka2	-0.071	1.0000	-0.688	0.9939	-0.974	0.9559	-0.414	0.9998
Ka1 - Ka3	0.111	1.0000	-2.997	0.0291*	-2.781	0.0549	-0.784	0.9869
Ka2 - Ka3	0.161	1.0000	-1.989	0.3492	-1.364	0.7882	-0.299	1.0000
LfL - Lf1	-0.154	1.0000	-2.089	0.2895	-1.677	0.5690	-1.524	0.6818
LfL - Lf2	3.873	0.0011**	-4.736	< 0.001***	-0.832	0.9815	-2.483	0.1214
Lf1 - Lf2	4.616	< 0.001***	-2.274	0.1971	0.820	0.9829	-1.006	0.9479
LzL - Lz1	5.114	< 0.001***	-6.470	< 0.001***	-1.777	0.4961	-4.889	< 0.001***

E

system comparison	relative total egg-spot area	
	z-value	p-value
Chitili - Kalambo	-0.143	0.9989
Chitili - Lufubu	3.378	0.0045**
Chitili - Lunzua	1.457	0.4550
Kalambo - Lufubu	4.712	< 0.001***
Kalambo - Lunzua	2.209	0.1184
Lufubu - Lunzua	-2.195	0.1223

Table S4. Results of the principal component analysis (PCA) testing the differences among males with regard to combined egg-spot characteristics number, relative average area, relative total area and coloration. Indicated are standard deviation, proportion of variance, cumulative variance and the mean of the PC loadings per population.

	PC1	PC2	PC3	PC4
Standard deviation	1.349000	1.123100	0.892800	0.348550
Proportion of Variance	0.455000	0.315300	0.199300	0.030370
Cumulative Proportion	0.455000	0.770300	0.969600	1.000000
ChL	0.898797	0.392387	-0.232222	0.061097
Ch1	-0.172236	-0.451941	0.528927	-0.075866
KaL	0.354268	0.037904	-0.119336	-0.089378
Ka1	0.693357	-0.253529	0.099019	-0.011017
Ka2	0.445778	-0.089104	0.158584	-0.002847
Ka3	-0.092053	0.002139	-0.016620	0.071001
LfL	0.226920	0.544469	0.144157	-0.110003
Lf1	-0.567835	0.884261	0.261697	-0.054627
Lf2	-1.290450	-0.349412	-0.016046	-0.057009
LzL	0.877900	0.459667	-0.414205	0.065597
Lz1	-1.279061	-0.497085	-0.256797	0.224243

Table S5. Orange ratio values for each depth level at the sample locations. The last column describes the average change in orange ratio per 10 cm, which was calculated from the deepest possible measurement (in bold). This average orange ratio was used in the analyses as a representative value for the underwater ambient light at each location.

locality	surface	10 cm	20 cm	30 cm	40 cm	50 cm	70 cm	100 cm	average (per 10 cm)
ChL	3.75	5.86	6.17	6.56	7.17	**7.56**	NA	NA	1.51
Ch1	3.59	6.06	**7.03**	NA	NA	NA	NA	NA	3.51
KaL	2.29	2.46	2.43	2.38	2.51	2.71	3.05	**3.41**	0.34
Ka1	2.82	3.37	3.56	3.77	4.09	4.46	5.30	**8.61**	0.86
Ka2	3.22	3.57	3.86	4.27	4.84	5.43	**10.02**	NA	1.43
Ka3	2.95	3.49	3.94	4.67	5.68	**7.64**	NA	NA	1.53
LfL	3.21	3.45	3.69	4.04	4.44	4.98	6.88	**10.40**	1.04
Lf2	3.02	3.60	4.33	**5.23**	NA	NA	NA	NA	1.74
LzL	2.45	2.57	2.67	2.71	2.78	2.81	2.94	**3.48**	0.35
Lz1	2.90	4.29	4.72	4.99	5.65	**6.25**	NA	NA	1.25

Table S6. Stepwise multiple regression on distance matrices (MRM) among pairwise differences in egg-spot characteristics, orange ratio, lymphocyte ratio as well as geographic distance. The egg-spot characteristics number, relative average area and coloration were corrected on centroid size before the analyses.

model		r^2-value	p-value
number	~ orange ratio + lymphocyte ratio + geographic distance	0.3729	0.0730
	~ orange ratio + lymphocyte ratio	0.2790	0.0580
	~ orange ratio + geographic distance	0.0501	0.5689
	~ lymphocyte ratio + geographic distance	0.3436	0.0417
	~ orange ratio	0.0007	0.8875
	~ lymphocyte ratio	0.2535	0.0102
	~ geographic distance	0.0498	0.1958
relative average area	~ orange ratio + lymphocyte ratio + geographic distance	0.5649	0.0573
	~ orange ratio + lymphocyte ratio	0.5245	0.0151
	~ orange ratio + geographic distance	0.0848	0.4083
	~ lymphocyte ratio + geographic distance	0.5279	0.0540
	~ orange ratio	0.0349	0.4330
	~ lymphocyte ratio	0.4902	0.0103
	~ geographic distance	0.0657	0.1489
relative total area	~ orange ratio + lymphocyte ratio + geographic distance	0.2428	0.1440
	~ orange ratio + lymphocyte ratio	0.0018	0.9789
	~ orange ratio + geographic distance	0.3995	0.0088
	~ lymphocyte ratio + geographic distance	0.2416	0.0688
	~ orange ratio	0.0633	0.2165
	~ lymphocyte ratio	0.0015	0.8887
	~ geographic distance	0.3826	0.0053
coloration	~ orange ratio + lymphocyte ratio + geographic distance	0.4493	0.0535
	~ orange ratio + lymphocyte ratio	0.4208	0.0460
	~ orange ratio + geographic distance	0.1024	0.2625
	~ lymphocyte ratio + geographic distance	0.1024	0.2625
	~ orange ratio	0.1001	0.0803
	~ lymphocyte ratio	0.0817	0.3902
	~ geographic distance	0.0003	0.9209

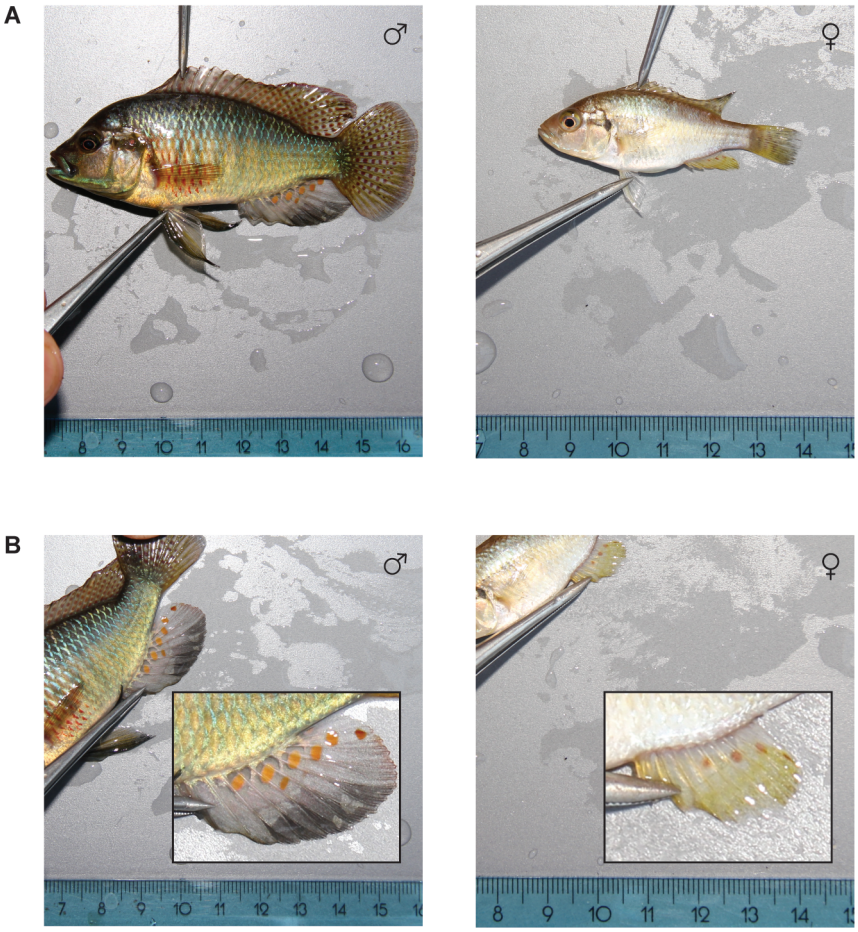

Figure S1. Photographs of a representative male (left side) and female (right side) in lateral position to measure centroid size (A) and focusing on the anal fin for later egg-spot measurements assessing the number, relative average area, relative total area and coloration (B).

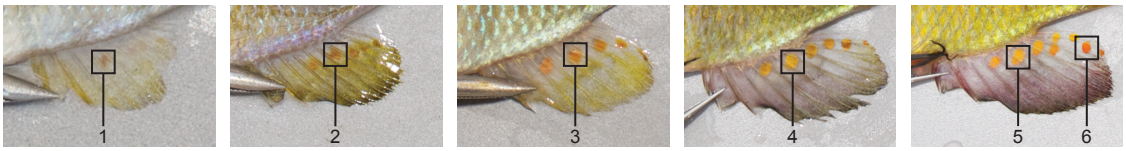

Figure S2. Representative photographs of the six categories used to describe the coloration of egg-spots. The categories ascend with increasing conspicuousness based on a combination of hue, saturation and brightness. 1 dull aggregated pigments; 2 dull egg-spot; 3 intermediate egg-spot; 4 normal egg-spot; 5 bright egg-spot (light orange); 6 bright and more saturated egg-spot (dark orange).

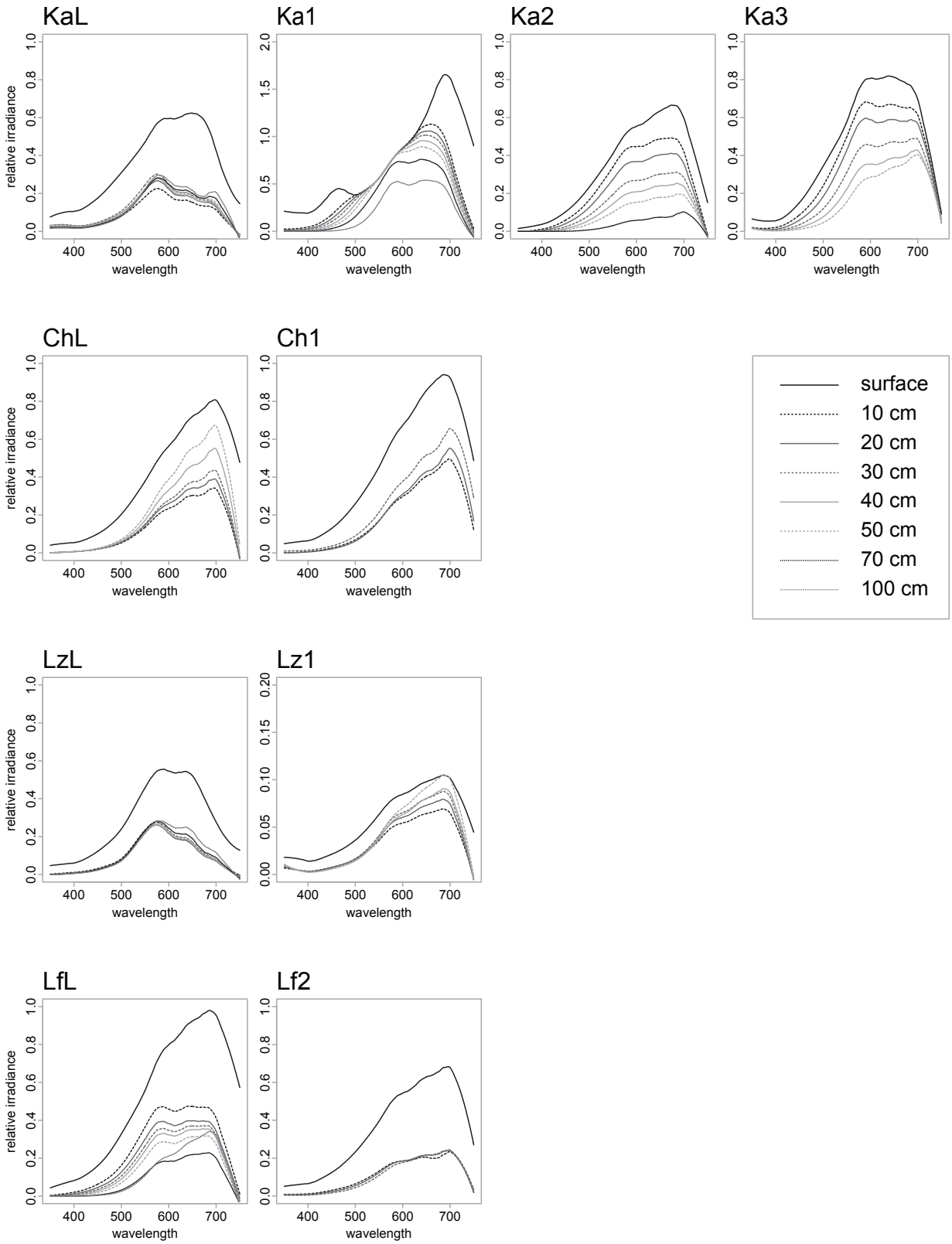

Figure S3. Underwater light environments. In each panel, the curves show underwater ambient light spectra at different depths.

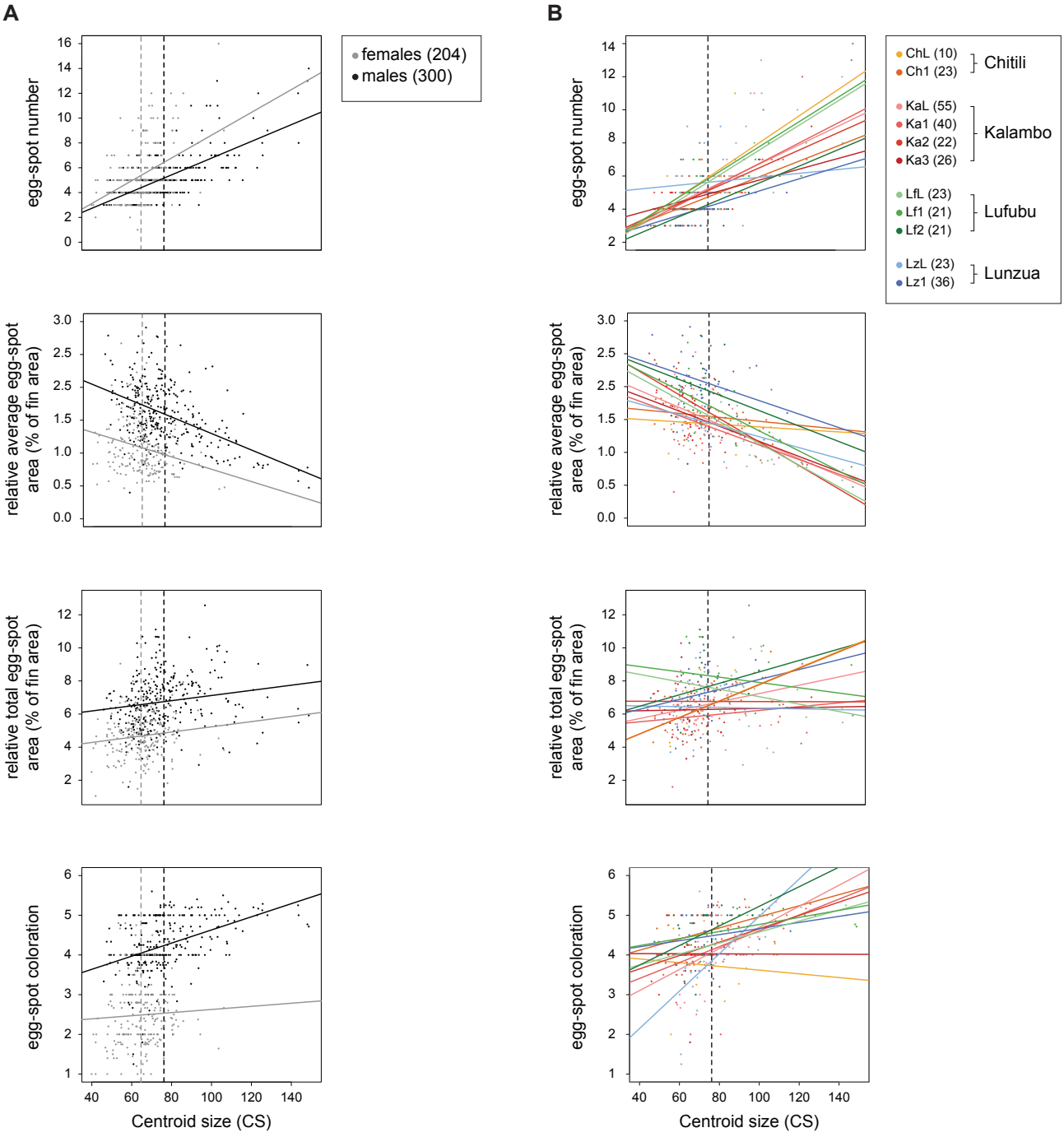

Figure S4. Correlations of body size and the four examined egg-spot characteristics measured based on photographs (number, relative average area, relative total area and coloration) for males and females (A) and for males of the populations of the lake-stream systems. Full names of the populations are listed in the grey box of Fig. 1.
The dashed vertical lines represent the mean value of body size (grey = females, black = males), which were used for the sex-specific centering and scaling of the data to compare males and females (A) and to correct for size in males to compare among populations (as illustrated in Fig. 2B) (B).

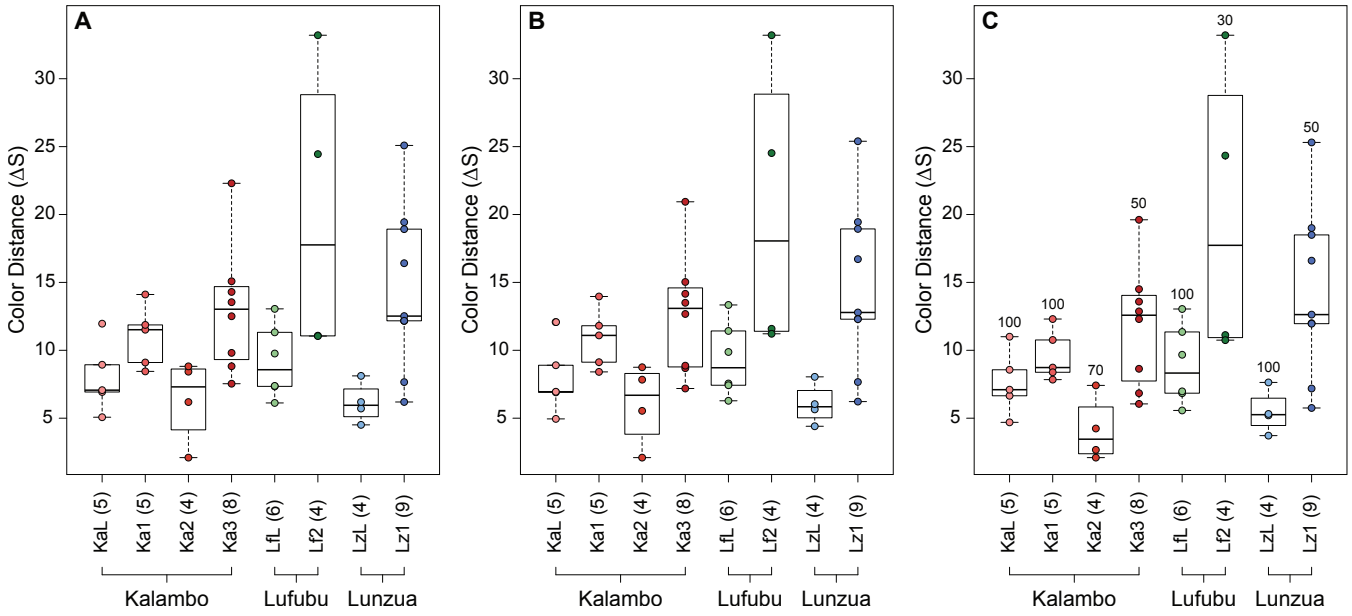

Figure S5. Color distances resulting from the visual models generated for 10 cm below water surface (A), 30 cm below surface (B) and for the deepest measurable depth for each locality (C; the corresponding depth is specified above the boxes). Corresponding sample sizes per population are parenthesized. For each system, populations are ordered on the x-axis with the lake populations on the left followed by the stream population(s).